Remarks

The parent PCT application includes claims 1-63, which were canceled at filing and replaced with claims 64-104. By subsequent supplemental preliminary amendment, claims 105-109 were added. In light of a Restriction Requirement, claims 96-104 and 109 are canceled herein without prejudice, as containing non-elected subject matter. Claims 64-95 and 105-108 are pending.

Response to Restriction

In accordance with 37 CFR §1.142(b), claims 96-104 and 109 have been cancelled herein as non-elected. Applicants reserve the right to pursue the cancelled subject matter in one or more divisional applications.

Objection to the Specification

The specification has been objected to as lacking the desired section headings. Applicants herewith submit a replacement specification that comprises the text of the parent published PCT (WO 00/28000) reformatted in accordance with current USPTO practice: (1) to include paragraph numbering; (2) to include cross-references to related applications; (3) to include a claim for foreign priority; (4) to include section headings; and (5) to include an Abstract. No new matter has been introduced. The nineteen pages of formal drawings submitted at filing are not replaced.

Claim Objections

Claim 69 was objected to for a typographic error, wherein the word --The-- was mistakenly typed as "he". Correction has been made.

Claim Rejections Under 35 USC §112, first paragraph

Claims 64-95 and 105-108 have been rejected as containing subject matter not described in the specification in such a manner as to enable the person of skill to practice the claimed invention. This rejection is based upon the lack of an Affidavit or Declaration by the Applicants or the Attorney of Record that the ESF116 mouse embryonic stem cell line has been deposited under the terms of the Budapest Treaty. As indicated at page 14, paragraph 58, the cell line has been

deposited under the terms of the Budapest Treaty, and the accompanying Affidavit, over the signature of the Attorney of Record, confirms and verifies the deposit and the ultimate availability of the cell line to the public upon grant of a patent derived from the present application.

Claims 65-95 and 105-108 have further been rejected as being non-enabled for the full breadth of the claim, and does not enable the person of skill to make and/or use the invention commensurate with the scope of the pending claims. This rejection is based generally on three issues: (1) not all ES cell lines may be permissive for the generation of esDC; (2) not all cytokines are capable of eliciting DC differentiation; and (3) not all inflammatory stimuli may induce maturation of esDC. This rejection is traversed for the reasons outlined below.

Issue 1: *ES cell lines*

Although the specification states (*see*, page 10, paragraph 42 of replacement specification) that esDC may be generated from ES cells derived from some but not all mouse strains, this statement is based upon one failed attempt to differentiate the 129.Sv ES cell line, R1. It is now known in the art that this result was the exception rather than the rule, and the cause has been identified as being the excessively high passage number of the R1 line. It is common knowledge among those involved in stem cell biology, that ES cell lines progressively lose the capacity to differentiate when maintained for long periods in culture, reflected in the number of times the cells have been passaged (*i.e.*, the passage number). Since R1 has been commercially available for many years, and has been passed from laboratory to laboratory during this time, the passage number has become excessively high, explaining the present inventors' inability to achieve DC differentiation. It is now common knowledge that other ES cell lines derived from the 129/Sv mice are perfectly capable of supporting esDC growth. Furthermore, *all* other lines, from a variety of strains, tested according to the present invention, have, without exception, proven permissive.

Moreover, there are at least two reasons why other laboratories culturing embryoid bodies in IL-3 and GM-CSF have not reported the production of DC. Firstly, these groups are not fully skilled in the culture and study of DC, having initiated experiments for the generation of cell types such as mast cells and B cells. Since the investigators' expertise lay elsewhere, it is unlikely that

they would have had the background knowledge to identify DC in their cultures; the means to assay their function; or the inherent interest in doing so. The lack of any discussion of DC in reports using IL-3 and GM-CSF does not, therefore, imply that DC were not present, indeed, any cultures of embryoid bodies yield many different cell types, not all of which can be described in every publication.

Secondly, it is clear that different cell types emerge from these cultures at different rates, the DC appearing comparatively late in the differentiation process, requiring several weeks of culture to accumulate to significant numbers. Since the differentiation of other cell types, such as erythrocytes, for example, is rather more rapid, it is possible that the investigators terminated their cultures long before DC had become the principle cell type.

Issue 2: *The combination of cytokines*

In the current Action, the Office correctly points out that ES cells will not differentiate into DC in response to *any* cytokine or combination of cytokines, which is entirely unsurprising and expected. The whole field of directed differentiation of ES cells depends on the capacity of distinct growth factor combinations to favor alternative differentiation pathways; it being incumbent on investigators to identify the combination required for development of the cell type in which they are interested. If every cytokine induced DC growth, the present invention would not be unexpected or surprising, which to the contrary, it is.

Issue 3: *Use of inflammatory mediators*

The capacity of DC to respond to inflammatory mediators and bacterial cell products, is wholly dependent on their expression of 'pattern recognition receptors' known as *Toll-like receptors* ("TLR"), of which nine family members have been identified. Importantly, populations of DC from distinct sources may express different subsets of TLR's, conferring on them the profile of stimuli to which they may respond. Since the literature is full of reports discussing patterns of TLR expression by distinct subsets of DC, no investigator skilled in the art would expect all DC to respond equally to every inflammatory stimulus. The burden of experimentation on the investigator skilled in the art is, however, greatly reduced, since, by knowing the pattern of TLR expression of

esDC, exact predictions may be made as to the mediators that would prove effective in inducing their maturation.

It is for all of the foregoing reasons that Applicants' believe the rejected claims to be fully enabled by the specification, and respectfully request that the rejections under §112, first paragraph be withdrawn.

Claim Rejections Under 35 USC §112, second paragraph

Claims 65-95 and 105-108 have been rejected as being indefinite. The rejection of all of the pending claims is based upon the rejection of claims 64, 65, 70 and 73; from which all of the remaining pending claims depend, either directly or indirectly. The rejection of each of the base claims is traversed for the following reasons.

Claim 64: *Rejected as being incomplete and vague*

The ability of immature DC differentiated from ES cells to produce long-term cultures lies in two properties which are not shared by DC from other sources and which are closely allied to their ancestry. The first is their lack of *spontaneous* maturation. Although esDC mature in response to inflammatory mediators and bacterial products, they do not do so in their absence, unlike DC derived from bone marrow precursors. Since, following maturation, DC display a limited life span, the lack of spontaneous maturation ensures highly protracted viability of esDC.

The other property that favors the derivation of long-term cultures is their pronounced capacity for self-renewal that greatly exceeds that of other populations. DC differentiated from ES cells may, therefore, be harvested routinely due to their ability to regenerate, thus contributing to the long-term nature of the cultures. Claim 64 has been amended to more clearly state the invention.

Furthermore, the term 'immunostimulatory phenotype' refers to the unique capacity of DC to stimulate primary responses among antigen-naïve T cells, a property that is not shared by any other population of antigen presenting cells. The acquisition of such an immunostimulatory capacity is known to occur as a direct consequence of maturation. The descriptive term

'immunostimulatory phenotype' is one that is widely used in the literature and would clearly and fully be understood by any investigator skilled in the art of DC culture.

Claims 65-95 and 105-108 depend directly or indirectly from claim 64, and are believed patentable for the same reasons as above applied to claim 64.

Claim 65: *Rejected as being unclear*

The phrase 'stimulating the immature DC to mature' refers to the addition to the culture medium of bacterial products or inflammatory mediators such as LPS. Since LPS acts as a stimulus for maturation by binding to *Toll-like receptor 4* ("TLR4") and transducing intracellular signaling cascades, the term 'stimulating' is technically correct in this context. However, in the interest of furthering prosecution of the application and obtaining allowance of the claims, claim 65 has been amended to replace the rejected term "stimulating" with the term --inducing--.

Claims 66-67 depend directly from claim 65, and are believed patentable for the same reasons as above applied to claim 65.

Claim 70: *Rejected as being incomplete*

The definition of embryoid bodies ("EB's") and protocols for their generation from ES cells are clearly and fully provided in the specification, for example, at page 9, paragraphs 37 and 38. However, in the interest of furthering prosecution of the application and obtaining allowance of the claims, claim 70 has been amended to include a statement of the protocol for generation of the embryoid bodies.

Claim 73: *Rejected as being vague*

The term 'immunomodulatory effect' encompasses any influence on progression of the immune response that alters the natural and expected outcome. An objective of the present invention is to be able to efficiently introduce heterologous genes into DC, which may subvert the subsequent immune response, either suppressing it in the case of autoimmune disease and allograft rejection, or heightening its potency, as would be desirable for the treatment of cancer and microbial infection. There would be little purpose in introducing genes in DC unless they were able to modulate the immunological function of the resulting cells.

Claims 74-75 and 77 depend directly or indirectly from claim 73, and are believed patentable for the same reasons as above applied to claim 73.

It is for all the foregoing reasons that Applicants' believe the rejected claims to fully satisfy the clarity requirement of 35 USC §112, second paragraph, and respectfully request that the rejections under this section be withdrawn.

There being no further issues, the application (including claims 64-95 and 105-108) is believed in condition for allowance, and such action is courteously requested.

Respectfully submitted,

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